Isozymes

IV

Genetics and Evolution

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GENE DUPLICATION WITHIN THE FAMILY SALMONIDAE:
II. DETECTION AND DETERMINATION OF THE GENETIC CONTROL OF DUPLICATE LOCI THROUGH INHERITANCE STUDIES AND THE EXAMINATION OF POPULATIONS

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ABSTRACT. We examine the interpretation of electro-
phoretic data reflecting duplicated gene loci in salmonid
fishes. Genetic models are considered to explain electro-
phoretic patterns of proteins reflecting duplicated
genes where common alleles give rise to proteins of
identical electrophoretic mobilities. It is shown that
in the absence of breeding data it is impossible to
distinguish between a model of tetrasomic inheritance
and one of disomic inheritance where alleles segregating
at two loci occur at the same frequency. However, it
is shown that disomic inheritance can be verified by
the examination of population phenotypic distributions
under certain conditions. Family data are presented
verifying disomic inheritance of malate dehydrogenase
in rainbow trout (Salmo gairdneri) and aspartate amino-
transferase in chum salmon (Oncorhynchus keta).
Examination of 19 salmonid biochemical systems indicate
that 8 systems clearly reflect gene duplication, 8 do
not, and 3 systems lack variation, precluding detection
of gene duplication; genetic variants at all of the
duplicated loci appear to segregate disomically. No
significant evidence for genetic linkage was found among
10 jointly segregating pairs of loci in rainbow trout
and one pair of loci in chum salmon; one comparison
in rainbow trout (IDH-AGPDH) approached significance
(P=.06).

INTRODUCTION

Measuring the patterns and amounts of genetic variation
in natural populations of many species is presently a major
thrust of experimental population genetics. This examination
has only been possible since the development of the electro-
phoretic separation of proteins as a means of examining the

gene products of many individual loci. Since 1968, our
group has conducted an extensive survey of genetic variation
in populations of fish and marine invertebrates. Our efforts
have been concentrated somewhat on the salmonid fishes because
of our interest in the evolutionary implications of their
extensive gene duplication and their usefulness in inheritance
studies. (The ripe sex products from both sexes can be
artificially removed and stored under refrigeration while the
parental types are determined electrophoretically prior to
selecting the desired matings.)

The efforts of one author (Allendorf) have been con-
centrated on an intensive survey of genetic variation in
populations of *Salmo* species with particular emphasis on
the rainbow trout (*S. gairdneri*). The extensive gene
duplication found in salmonids often confounds the genetic
interpretation of the observed variation. Because of the
critical importance of understanding this genetic basis we
are involved in a series of inheritance studies directed
toward the identification of the genetic control of polymor-
phic loci in salmonids.

The initial electrophoretic studies with salmonids
revealed additional loci coding for lactate dehydrogenase
(LDH) in comparison with other vertebrate species (Morrison
and Wright, 1966). Subsequent studies of salmonids have
revealed similar gene duplications for a number of other
enzymes--e.g. malate dehydrogenase (Bailey et al., 1970) and
isocitrate dehydrogenase (Wolf et al., 1970). Ohno has
postulated that the salmonids are descended from a recent
tetraploid ancestor based upon this gene duplication, relative
DNA contents, and chromosomal characteristics (Ohno et al.,
1969). Although Ohno's theory is sound and based upon valid
evidence, some subsequent attempts to reinforce this theory
have not been sound (see Allendorf and Utter, 1973). Upon
reading the relevant literature one is left with the im-
pression that virtually all enzymes in salmonids are coded
for by duplicate loci and that many of these loci are
segregating tetrasomically. Our studies are in conflict with
this impression. We have found no evidence for the duplicat-
ion of many protein loci and we have found no indication of
tetrasomic inheritance even for those loci which have been
reported to be inherited tetrasomically (this in no way
rules out the existence of tetrasomic loci in salmonids
but rather points out that they have not yet been detected).

The purpose of this present paper is to examine the
interpretation of duplicate loci detected electrophoretically
and to review our genetic investigations of rainbow trout
in view of the accepted level of gene duplication and

patterns of inheritance of salmonid loci. The evolutionary
question to be considered in the course of these studies is
the fate of duplicated loci. We feel that the salmonids
represent an outstanding opportunity to examine this question
experimentally.

DETERMINATION OF GENETIC CONTROL OF DUPLICATED LOCI

The multiple loci coding for many salmonid proteins
often make the interpretation of genetic control rather
difficult. The disagreements which can result are illustrated
by the reported number of loci coding for LDH in salmonids;
values all the way from five to eight have been reported in
the literature (Massaro and Markert, 1968).

We will examine a hypothetical case of a single enzyme
and outline some of the difficulties in determining the
exact nature of genetic control of that enzyme. We will
restrict our example to the case of a maximum of two disomic
loci (or a single tetrasomic locus) with two alleles (A and
A'). Table 1 outlines possible genetic models controlling
these loci, while Figure 1 presents typical electrophoretic
phenotypes for a dimeric enzyme in this hypothetical case.
Two basic questions are to be examined: (1) Is there any
evidence of gene duplication? and (2) If there has been
gene duplication, is there a single tetrasomic locus or two
disomic loci?

PHENOTYPIC DISTRIBUTIONS IN POPULATIONS

Predictions of the expected phenotypic distributions
for each of the models can be made assuming Hardy-Weinberg
proportions--see Table 2. Certain conclusions can be
drawn pertaining to the nature of genetic control involved
by comparing the observed distributions within populations
with these theoretical distributions.

Models A, C, and G all predict a single-banded phenotype
to be found in all individuals of a population because of
the lack of polymorphism. Therefore, no conclusions of
genetic control can be made if this distribution is found in
a population. However, model D which also lacks polymorphism
predicts a fixed multi-banded phenotype to be found in all
individuals. If this "fixed heterozygote" situation is found
in a population it is best explained by two monomorphic,
disomic loci fixed for alleles with differing electrophoretic
mobilities.

Model B is also distinguishable in that there are no
asymmetrical banded phenotypes and the single banded alter-

TABLE 1

List of the possible genetic models to be considered that
could be controlling a protein system examined electro-
phoretically. (P = frequency of allele A; Q = frequency of
allele A'.)

Genetic Model	Disomic Loci		Tetrasomic Loci		Allele Frequencies
	Mono-morphic	Poly-morphic	Mono-morphic	Poly-morphic	
A	1	0	0	0	$p=1$
B	0	1	0	0	p
C	2	0	0	0	$p_1=p_2=1$
D	2	0	0	0	$p_1=1, p_2=0$
E	1	1	0	0	$p_1=1, p_2$
F	0	2	0	0	p_1, p_2
G	0	0	1	0	$p=1$
H	0	0	0	1	p

nate homozygote type is found at a much higher frequency
than under any of the other models.

Models E, F, and H are not so easily distinguished. In
fact, Models F (two polymorphic disomic loci) and H (a single
polymorphic tetrasomic locus) predict identical phenotypic
distributions when $q_1=q_2$ for model F. Therefore, the exis-
tence of tetrasomic inheritance can never be conclusively
demonstrated by examining the phenotypic distribution in
a population since a disomic model can always fit the data
equally well. When there is a comparatively high amount of
variation model E is distinguishable from models F and H by
virtue of the absence of phenotypes four and five. Accord-
ingly, when the amount of variation is low these models are
not easily distinguished. It is interesting to examine the
predicted phenotypic distributions of these three models as
shown in Figure 2. The expected Hardy-Weinberg proportions
of a population are represented by the family of values
between these two curves in the case where the correct model
cannot be distinguished. Similarly, if Model F is shown
to be the correct one (through inheritance studies as outlined

TABLE 2

Expected Hardy-Weinberg proportions for each of the eight genetic models considered as outlined in Table 1.

<u>Expected Phenotypic Distributions</u>
Genetic Model

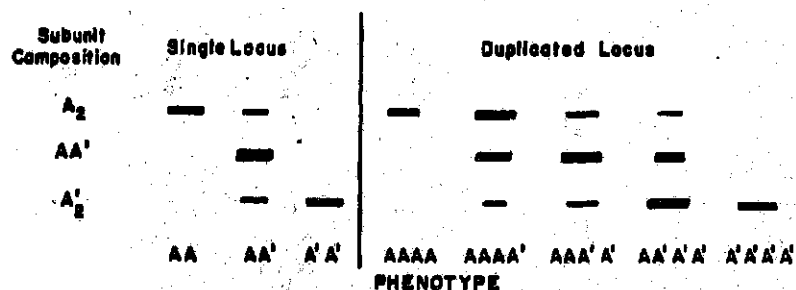| Duplicated Phenotype	Non-Dupl Phenotype	A	B	C	D	E	F	G	H
A_4	A_2	1	p^2	1	0	p_2^2	$p_1^2 p_2^2$	1	P
A_3A'	---	0	0	0	0	$2p_2 q_2$	$2p_1^2 p_2 q_2 + 2p_1 p_2^2 q_1$	0	$4P^3 Q$
$A_2A'_2$	AA'	0	$2pq$	0	1	q_2^2	$p_1^2 q_2^2 + 4p_1 p_2 q_1 q_2 + p_2^2 q_1^2$	0	$6P^2 Q^2$
AA'_3	---	0	0	0	0	0	$2p_1 q_1 q_2^2 + 2p_2 q_1^2 q_2$	0	$4PQ^3$
A'_4	A'_2	0	q^2	0	0	0	$q_1^2 q_2^2$	0	Q^4

Figure 1. Diagrammatic representation of typical electrophoretic phenotypes for a dimeric enzyme controlled by either a single locus or a duplicated locus.
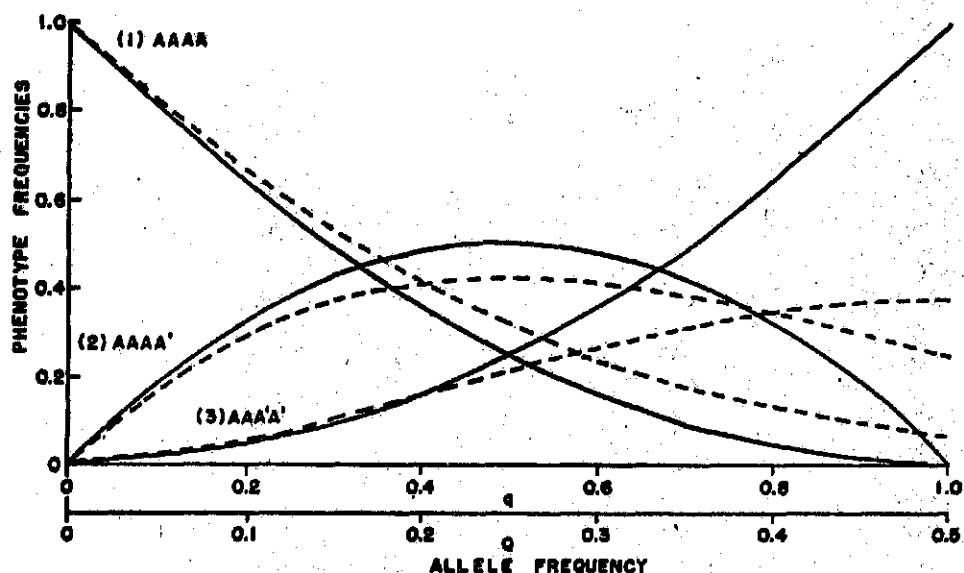

Fig. 2. Graphical representation of expected phenotypic distributions for Model E(solid line) and Models F and H (dotted line) when $q_1=q_2$ under Model F. Upon examining a population, the overall allele frequency (Q) of the A' allele can be determined by dividing the total number of A' alleles observed by 4N (where N=number of individuals examined). This frequency is the allele frequency for a tetrasomic locus

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Legend for Fig. 2, continued:
(Q) and is also equal to both q_1 and q_2 $(Q=q_1=q_2)$ under
Model F when $q_1=q_2$. This frequency is related to q_2 from
Model E by the equation $q_2=2Q$. This graph demonstrates the
differences in expected Hardy-Weinberg proportions for a
duplicated locus for these three cases; (1) a single poly-
morphic locus, (2) both loci polymorphic and (3) a single
tetrasomic locus. When $q_1 \neq q_2$ for case (2) the expected
Hardy-Weinberg proportions lie between these curves.

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in the next section), the expected Hardy-Weinberg proportions
are represented by this same family since gene frequencies
cannot be assigned to individual loci.

The major conclusions that can then be drawn from this
section are that (1) tetrasomic inheritance cannot be
conclusively demonstrated only by the phenotypic distribution
of a population and (2) under certain conditions, disomic
inheritance can be demonstrated in this way.

INHERITANCE EXPERIMENTS

The mode of inheritance of duplicated loci (either
disomic or tetrasomic can be positively verified through
inheritance studies. Individuals with single doses of a
variant allele do not provide useful information since their
gametes are expected to segregate 1:1 (AA : AA') under
both a tetrasomic and disomic model. However, individuals
with a double dose of a variant allele do provide sufficient
information to distinguish between these models.

Examination of the gametic output from an individual
typed as AAA'A' will provide the desired information. The
phenotype AAA'A' can represent any one of three genotypes.

Genotype I (Disomic)	A	A'
$A_1A_1A_2'A_2'$	A	A'
Genotype II (Disomic)	A	A
$A_1A_1'A_2A_2'$	A'	A'
Genotype III (Tetrasomic)	A	
	A	
AAA'A'	A'	
	A'	

These genotypes and their expected gametic ratios are presented in Table 3. It can be seen that each genotype predicts a unique gametic distribution. In the disomic case, the proportion of genotypes I and II reflect the gene frequencies p_1 and p_2. The gametic ratios for genotypes II and III are variable because of possible linkage in the disomic model and possible double reduction division in the tetrasomic model. Therefore, to verify disomic inheritance, parental types corresponding to both genotypes I and II should be observed. In addition, it should be noted that examining the gametic ratios from genotype II will also provide a measure of possible linkage between the two loci.

MATERIALS AND METHODS

The results presented here are based on the examination of thousands of rainbow trout from numerous populations as well as from a continuing series of experimental matings. We have examined both non-anadromous and anadromous (steelhead) populations of *S. gairdneri*, and have found no reason for treating these two forms as being distinct. For purposes of this paper, we will use the common name rainbow trout to represent both the anadromous and non-anadromous forms.

Our methods have been documented elsewhere (Utter et al., 1973a). Three buffer systems were used: (1) a discontinuous system described by Ridgway et al., (1970), (2) a continuous tris-borate-EDTA system (pH 8.6) described by Markert and Faulhaber (1965), and (3) a continuous phosphate system (pH 6.5) described by Wolf et al., (1970).

The following proteins were examined in the course of these studies: AAT (aspartate aminotransferase); ADH (alcohol dehydrogenase); AGPDH (alphaglycerophosphate dehydrogenase); DIA (diaphorase); EST (esterase); IDH (isocitrate dehydrogenase); LDH (lactate dehydrogenase); MDH (malate dehydrogenase); ME (malic enzyme); 6PGDH (6-phosphogluconate dehydrogenase); PGM (phosphoglucomutase); SDH (sorbitol dehydrogenase); TFN (transferrin); TO (tetrazolium oxidase); and XDH (xanthine dehydrogenase). All of these proteins migrated anodally with the exception of ADH.

The mating experiments presented for MDH-B and AAT were carried out in the 1973-1974 spawning season. The progeny from these matings are still being examined. A more detailed report of the inheritance of these loci will be presented elsewhere.

TABLE 3
Comparison of disomic verus tetrasomic inheritance in an
individual carrying two doses of a variant allele.

PARENTAL GENOTYPE

(1)	(2)	(3)

$A_1A_1A'_2A'_2$ $A_1A'_1A_2A'_2$ $AAA'A'$

COMPARATIVE SEGREGATION RATIOS OF GAMETES

Gamete Genotype	Parental Genotype		
	(I)	(II)*	(III)**
AA	0	1	1
AA'	1	2	4
A'A'	0	1	1

* Assuming no linkage.

** Assuming chromosome segregation.

RAINBOW TROUT PROTEIN LOCI

DIA, 6PGDH, XDH. These enzymes were found to be represented by a single invariant band in all individuals examined. Since this result is in agreement with models A, C, and G, no conclusions can be made as to whether they are coded for a single locus or duplicated loci.

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ME. Two forms of this enzyme were observed--one which was predominant in muscle extracts and one which was predominant in liver extracts. Both of these forms were uniformly seen as a single band. As explained above, the number of loci coding for each form could therefore not be determined.

ADH. The great majority of individuals examined also displayed a single invariant band for this enzyme. A few variant types with a more cathodal allele have been seen however. In these variant individuals, there is no indication of any asymmetrical banding intensities as would be expected for a duplicated locus. For this reason ADH is assumed to be coded for by a single disomic locus.

PGM, TO, TFN. These enzymes have all been previously reported by our laboratory to be represented by single disomic polymorphic loci in rainbow trout (Utter and Hodgins, 1972). A report of possible gene duplication of TO (Cederbaum and Yoshida, 1972) was found to be in error (Utter et al., 1973b).

LDH. Our previously reported results (Utter et al., 1973a) indicate five loci coding for LDH in salmonids. This enzyme is an example of fixed heterozygosity in that all five loci have common alleles with differing mobilities. The duplicated LDH-B loci present an especially interesting evolutionary history. In addition to evolving common alleles of different mobilities, these loci have also evolved differential tissue specificity. Only the B_2 locus is expressed in liver tissue while the B_1 locus is strongly predominant in heart tissue.

Although LDH is the classic example of duplicate loci in salmonids, not all salmonid LDH loci display gene duplication. The eye form of LDH is present in many families of fish and is presumed to be the result of a single evolutionary event (Horowitz and Whitt, 1972). This indicates that the LDH eye form was present in the salmonid lineage before the presumed polyploid event. However, genetic evidence has been presented which shows the salmonid eye LDH to be coded by a single locus (Morrison and Wright, 1966; Wright and Atherton, 1970).

AGPDH. Previous publications from our group have reported this enzyme to be represented by a single polymorphic disomic locus (Utter and Hodgins, 1972). Examination of this enzyme with buffer system (3) clearly revealed the

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presence of an additional locus not detectable with buffers
(1) or (2). The gene products of this second locus do
interact with the products of the first locus in that the
appropriate heterodimers are formed. This additional locus
is fixed for an allele with a different mobility than both
alleles seen at the first locus, thereby creating a fixed
heterozygote effect.

EST. A single polymorphic disomic locus predominantly
expressed in liver extracts has been observed. Inheritance
studies have confirmed the genetic basis of this variation
but there are indications of possible ontogenetic and envir-
onmental effects on the expression of this locus. There is
no indication of duplication of this locus.

SDH. This enzyme has been reported to be tetrasomically
inherited in rainbow trout on the basis of the observed
phenotypic distribution in a single population (Engel et al.,
1970). As shown in a prior section, tetrasomic inheritance
cannot be verified in this manner. We have found multiple
banded phenotypes in all rainbow trout examined in accordance
with model D, indicating this enzyme to be coded for by two
disomic loci with common alleles of different mobilities.
We have seen genetic variation at low frequency for this
enzyme but have not found it in the populations used for
the mating experiments. However, we do have preliminary
inheritance data for a similar variant found in cutthroat
trout (*S. clarki*) which verifies this interpretation.

IDH. The supernatant form of this enzyme (IDH-s) has
also been reported to be inherited tetrasomically in rain-
bow trout on the basis of the phenotypic distribution in a
population (Wolf et al., 1970). In an inheritance study
previously reported (Allendorf and Utter, 1973), we have
shown this variation to be controlled by two disomic loci.
    The mitochondrial form of this enzyme (IDH-m), as best
seen in muscle extracts, is represented by three nonvariant
bands indicating the presence of two monomorphic disomic loci
with common alleles of different mobilities.

MDH-B. Bailey et al., (1970) presented an excellent
biochemical and genetic analysis of salmonid MDH demonstrat-
ing the existence of duplicate loci coding for the B form.
However, since all of their matings involved individuals
with at most a single dose of the variant allele they could
not distinguish between disomic and tetrasomic inheritance.
We have recently made the necessary crosses to answer this

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TABLE 4

Inheritance of duplicate loci. In each case only the results from one family are presented here. Additional families (some with parental types corresponding to genotype 1 in Table 3) further confirm these conclusions.

(a) Rainbow Trout MDH-B

		PROGENY PHENOTYPES OBSERVED (DISOMIC) /TETRASOMIC/						
PARENTAL PHENOTYPES		BBBB	BBBB'	BBB'B'	x^2	d.f.		P
BBBB	BBB'B'	80	165	70				
		(78.8)	(157.5)	(78.8)	1.36	2	>	0.5
		/52.5/	/210.0/	/52.5/	33.36	2	<	0.0001

(b) Chum Salmon AAT

		PROGENY PHENOTYPES OBSERVED (DISOMIC) /TETRASOMIC/						
PARENTAL PHENOTYPES		AAAA	AAAA'	AAA'A'	x^2	d.f.		P
AAAA	AAA'A'	44	85	43				
		(43.0)	(86.0)	(43.0)	0.03	2	>	0.9
		/28.7/	/114.7/	/28.7/	27.80	2	<	0.0001

TABLE 4, continued

Conclusions for both cases:

(1) Inherited disomically

(2) Parental genotypes - (a) $B_1B_1B_2B_2$ X $B_1B_1'B_2B_2'$

(b) $A_1A_1A_2A_2$ X $A_1A_1'A_2A_2'$

(3) No indication of linkage

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question. Table 4 outlines the results from one of these crosses. There are clearly two disomic loci both of which are polymorphic in the population we examined. Additional results from other families confirmed this conclusion. In addition, Table 4 examines possible linkage between the two loci by comparing the frequencies of the two linkage classes (BBBB + BBB'B' : BBBB'). There is no indication of linkage on this basis.

MDH-A. Bailey et al., (1970) presented some evidence for the duplication of this locus in brown trout (*S. trutta*) and subsequent evidence has supported this conclusion (Bailey, personal communication). We have seen a variant of this form at a low frequency in some rainbow trout populations. Based on the relative intensities of bands in the variant phenotypes it appears that this locus is not duplicated in rainbow trout. Additional evidence in the form of inheritance data is desirable before it can be firmly concluded that this enzyme is coded for by different number of loci in these two closely related species.

AAT. The common phenotype found in rainbow trout for this enzyme is a single distinct band. There is a low frequency variant found in some populations. The intensities of bands found in the variant phenotypes are typical of a duplicated locus. Inheritance studies have not been carried out for this enzyme with rainbow trout. However, inheritance studies have been done for this enzyme with the chum salmon (*Oncorhynchus keta*). The results from one family from these experiments are shown in Table 4. Comparison with the expected disomic and tetrasomic ratios indicate that chum salmon AAT is controlled by two polymorphic disomic loci.

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As with rainbow trout MDH-B loci, there is no indication of linkage between these duplicated loci. One cannot conclude on this basis that a similar mode of genetic control exists in rainbow trout. However, these data directly apply to the question of genetic control of duplicated loci in salmonids.

JOINT SEGREGATION OF LOCI

Whenever possible in the course of our inheritance experiments, we have tested for linkage between segregating loci by testing for any aberrant 2-way joint segregation in progeny from double heterozygote individuals. The results of these tests are summarized in Table 5. There is no definite indication of linkage. In the case of IDH - AGPDH, the deviation from random segregation did approach significant proportions (P > .06). Unfortunately, it has not been possible to examine further the joint segregation of these loci.

Of particular interest is the joint segregation of duplicated loci. Aberrant joint segregation of duplicated LDH loci in both brook trout (*Salvelinus fontinalis*) and rainbow trout have been reported (Morrison, 1970; Davisson et al., 1973). Recently, Aspinwall (1974) has reported limited data indicating aberrant joint segregation of duplicated MDH loci in pink salmon (*O. gorbuscha*). Our results with MDH-B in rainbow trout and AAT in chum salmon do not indicate any abnormal joint segregation of these duplicated loci in these species.

SUMMARY

Table 6 presents a summary of the rainbow trout loci we have examined. Contrary to the statements of some authors, a significant portion of loci in this salmonid species show no evidence of gene duplication. If we accept the tetraploid origin of salmonids, these loci then seem to represent evidence of Haldane's (1933) original suggestion that if a gene is duplicated one of the two resulting genes may become nonfunctional because of the fixation of a deleterious mutation. One other possible fate of a duplicated gene is the evolution of differential function as demonstrated by the duplicated LDH-B loci which have evolved differential tissue specificity.

We have found no evidence of tetrasomic inheritance in salmonids. We have also shown that previous reports of tetrasomic inheritance in salmonids are not sound. These results do not, of course, rule out the existence of tetrasomic loci in salmonids, but rather are meant to show that there is

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TABLE 5

Joint segregation ratios of rainbow trout (and chum salmon) loci.

Loci		Linkage Class I	Linkage Class II	Chi-square*
MDH-B$_1$	IDH-3	62	55	.42
		34	23	2.12
TO	IDH-3	69	50	3.03
		52	46	.37
MDH-B$_1$	TO	40	53	1.82
		71	76	.17
		89	79	.60
MDH-B$_1$	AGPDH-1	73	72	.01
LDH-B$_2$	IDH-3	66	59	.39
LDH-B$_2$	TO	53	57	.15
		86	74	.90
		100	108	.31
LDH-B$_2$	MDH-B$_1$	53	44	.84
		48	52	.16
		121	127	.15
TO	AGPDH-1	70	79	.54
IDH-3	AGPDH-1	49	70	3.71
MDH-B$_1$	MDH-B$_2$	150	165	.71
**AAT-1	AAT-2	85	87	.02

* - d.f. = 1
** - Chum salmon

TABLE 6.
Summary of rainbow trout loci examined.

Protein	Duplicated?	Loci	Mode of inheritance (if duplicated)
AAT	Yes	AAT-1	(Disomic in chum salmon)
		AAT-2	
ADH	No	ADH	
AGPDH	Yes	AGPDH-1	Disomic
		AGPDH-2	
DIA	?	DIA	
EST	No	EST	
IDH(m)	Yes	IDH-1	Disomic
		IDH-2	
IDH(s)	Yes	IDH-3	Disomic
		IDH-4	
LDH-A	Yes	$LDH-A_1$	Disomic
		$LDH-A_2$	
LDH-B	Yes	$LDH-B_1$	Disomic
		$LDH-B_2$	
LDH-C	No	LDH-C	
MDH-A	No	MDH-A	
MDH-B	Yes	$MDH-B_1$	Disomic
		$MDH-B_2$	
ME	No	ME-1	
		ME-2	
6PGDH	?	6PGDH	
PGM	No	PGM	

TABLE 6, continued:

Protein	Duplicated?	Loci	Mode of in-heritance (if duplicated)
SDH	Yes	SDH-1 SDH-2	Disomic
TFN	No	TFN	
TO	No	TO	
XDH	?	XDH	

at present no evidence for tetrasomic inheritance in salmonids.

REFERENCES

Allendorf, F.W. and F.M. Utter 1973. Gene duplication within the family Salmonidae: Disomic inheritance of two loci reported to be tetrasomic in rainbow trout. *Genetics* 74: 647-654.

Aspinwall, N. 1974. Genetic analysis of duplicate malate dehydrogenase loci in the pink salmon, *Oncorhynchus gorbuscha*. *Genetics* 76: 65-72.

Bailey, G.S., A.C. Wilson, J.E. Halver, and C.L. Johnson 1970. Multiple forms of supernatant malate dehydrogenase in salmonid fishes. *J. Biol. Chem.* 245: 5927-5940.

Cederbaum, S.D. and A. Yoshida 1972. Tetrazolium oxidase polymorphism in rainbow trout. *Genetics* 72: 363-367.

Davisson, M.T., J.E. Wright, and L.M. Atherton 1973. Cytogenetic analysis of pseudolinkage of LDH loci in the teleost genus *Salvelinus*. *Genetics* 73: 645-658.

Engle, W., J. Op't Hof, and U. Wolf 1970. Genduplikation durch polyploide Evolution: die Isoeyzyme der Sorbit dehydrogenase bei herings-und lachsartigen Fischen (Isospondyli). *Humangenetik* 9: 157-163.

Haldane, J.B.S. 1933. The part played by recurrent mutation in evolution. *Amer. Nat.* 67: 5-19.

Horowitz, J.J. and G.S. Whitt 1972. Evolution of a nervous system specific lactate dehydrogenase isozyme in fish. *J. Exp. Zool.* 180: 13-32.

Markert, C.L. and I. Faulhaber 1965. Lactate dehydrogenase isozyme patterns of fish. *J. Exp. Zool.* 159: 319-332.

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Massaro, E.J. and C.L. Markert 1968. Isozyme patterns of
    salmonid fishes: evidence for multiple cistrons for
    lactate dehydrogenase. *J. Exp. Zool.* 168: 223-238.
Morrison, W.J. 1970. Nonrandom segregation of two lactate
    dehydrogenase subunit loci in trout. *Trans. Amer. Fish.
    Soc.* 99: 193-206.
Morrison, W.J. and J.E. Wright 1966. Genetic analysis of
    three lactate dehydrogenase isozyme systems in trout:
    Evidence for linkage of genes coding subunits A and B.
    J. Exp. Zool. 163: 259-270.
Ohno, S.M., J. Muramato, J. Klein, and N.B Atkin 1969.
    Diploidtetraploid relationship in Clupeoid and Salmonid
    fish. *Chromosomes Today* 2: 139-147.
Ridgway, G.J., S.W. Sherburne, and R.D. Lewis 1970. Poly-
    morphism in the esterases of Atlantic herring. *Trans.
    Amer. Fish. Soc.* 99: 147-151.
Utter, F.M., F.W. Allendorf, and H.O. Hodgins 1973a. Genetic
    variability and relationships in Pacific salmon and
    related trout based on protein variations. *Syst.
    Zool.* 22: 257-270.
Utter, F.M., F.W. Allendorf, H.O. Hodgins, and A.G. Johnson
    1973b. Response to tetrazolium oxidase polymorphism
    in rainbow trout. *Genetics* 73: 159.
Utter, F.M. and H.O. Hodgins 1972. Biochemical genetic
    variation at six loci in four stocks of rainbow trout.
    Tran. Amer. Fish. Soc. 101: 494-502.
Wolf, U., W. Engel, and J. Faust 1970. Zum mechanismus der
    Diploidisierung in der Wirbeltier evolution: Koexistanz
    von tetrasomen und disomen Genloci der isocitrat-
    Dehydrogenasen bei der Regenbagen forelle (*Salmo
    irideus*). *Humangenetik* 9: 150-156.
Wright, J.E. and L. M. Atherton 1970. Polymorphism for LDH
    and transferrin loci in brook trout populations.
    Trans. Amer. Fish. Soc. 99: 179-192.